

of 700 kHz. The chamber between the IMS cell and quadrupole time-of-flight (Q-TOF) mass spectrometer was evacuated using a mechanical pump (Alcatel 2033, Adixen-Alcatel, Hingham, Mass.) to a pressure of ~300 mTorr. Once into the Q0 or collisional quadrupole of the modified commercial Q-TOF (MDS Sciex, Q-Star Pulsar, Concord, Canada), the ions were guided into the pulsing region of the Q-TOF operated at ~7 kHz, which spanned a mass range of 50-2000 m/z. The ion optics of the Q-TOF system were optimized to maximize ion transmission and signal intensity while minimizing the ion transit time to the detector. Data were recorded using a 10-GHz time-to-digital converter interfaced to a custom built software package. Timing sequence of the ion mobility experiment was synchronized with the pulsing frequency of the Q-TOF and controlled using a timing card.

Example 3

Peptide Analysis Using Electrodynamic Ion Funnel, IFT, and oa-TOF-MS

[0041] An electrodynamic ion funnel was coupled to the IFT and subsequently to an orthogonal acceleration-time-of-flight (oa-TOF) mass spectrometer in a prototype dual-stage reflectron oa-TOF mass spectrometer configuration (FIG. 5). Low concentration peptide mixtures were analyzed with the IFT in trapping mode. The IFT was coupled through use of added terminal electrodynamic ion funnel electrodes enabling control over the axial dc-gradient in the trapping portion of the IFT. Ions generated in an electrospray source were transmitted through a 508 μm inner diameter (i.d.), 10 cm long stainless steel capillary interface, resistively heated to 165° C., and into the IFT at pressure of ~1 Torr. The 180° phase-shifted rf-fields were applied to adjacent ring-electrodes at a peak-to-peak amplitude of 70 V_{p-p} and a frequency of 600 kHz. Ion transmission through the funnel was improved by superimposing a dc-field onto the rf-field applied to each electrode. In the continuous mode, the dc-gradient applied to the funnel was 20 V/cm. Measurements indicated a maximum charge capacity of ~3×10⁷ charges. An order of magnitude increase in sensitivity was observed. A signal increase in the trapping mode was accompanied by reduction in the chemical background. Controlling IFT ejection time resulted in efficient removal of singly charged species and improved the signal-to-noise ratio (S/N) for multiply charged analytes. The ion funnel and IFT combination consisted of 98 brass ring electrodes. Each electrode was 0.5 mm thick and was separated with TEFLON® (polytetrafluoroethylene) spacers 0.5 mm apart. The ion funnel which accepts ions exiting the heated capillary was composed of 24 ring electrodes. Inner diameters (i.d.) of the ring electrodes varied from 25.4 mm at the ion funnel entrance, 19.1 mm in the trapping portion of the IFT, and 2.4 mm at the exit electrode in the outlet portion of the IFT. A jet disrupter in the funnel reduced gas load to subsequent stages of differential pumping while maintaining high ion transmission. Ions exiting the ion funnel were introduced to the inlet portion of the IFT through a 3 mm conductance limit orifice and were accumulated in the trapping portion in trapping mode. The trapping portion comprised, e.g., 10 ring electrodes, each having an internal diameter (i.d.) of, e.g., 19.1 mm. The trapping portion of the IFT was separated from the inlet portion on the ion receiving side and the outlet portion on the ion exit side of the trapping portion by two electrostatic grids fabricated from commercially available 95%-transmission nickel mesh (InterNet Inc.,

Minneapolis, Minn.). Pulsing voltages applied to the electrostatic grids were used to control ion populations introduced into the IFT, as well as to control ion storage and extraction times, respectively. A dc-gradient in the trapping portion of the IFT was varied independently from the dc-gradient in the ion funnel by adjusting potentials at a first dc-electrode ("Trap in") and a last dc-electrode ("Trap out") in the trapping portion, described previously herein. In continuous mode, potentials on the electrostatic grids were optimized to ensure efficient ion transmission through the trapping region. Ions passing through the trapping portion were recollimated in the outlet (converging) portion and then focused into a 15 cm long collisional quadrupole operating at a pressure of ~6×10⁻³ Torr. After collisional relaxation and focusing, ions were transmitted through a 20 cm long selection quadrupole at a pressure of 1.5×10⁻⁵ Torr and focused by an Einzel lens assembly into a TOF extraction region. Collisional and selection quadrupoles were operated at an rf-amplitude of 2500 V_{p-p} and an rf-frequency of 2 MHz. The TOF chamber included a stack of acceleration electrodes, a dual-stage ion mirror, and a 40 mm diameter extended dynamic range bipolar detector, having a 10 μm pore size and 12°±1 bias angle (Burle ElectroOptics, Sturbridge, Mass.). Length of the TOF flight tube was 100 cm, and the distance between the center of the 40 mm long TOF extraction region and the detector axis was 75 mm. Typical full width at half-maximum (fwhm) of signal peaks were 3.0-3.5 ns, yielding an optimum resolving power of 10,000 and a routine resolving power of from 7,000-8,000. The TOF detector was impedance matched to a 2 GS/s 8-bit analog-to-digital converter that enabled routine mass measurement accuracy of ~5 ppm. Continuous and pulsed ion currents in the TOF acceleration stack were measured using a Faraday cup charge collector positioned on the interface axis immediately downstream of the TOF extraction region. Ion current pulses were acquired using a fast current inverting amplifier coupled to a digital oscilloscope. Pulse sequencing for ion trapping was used. With one of the TOF MS control bits (Run/Stop) toggled high at the beginning of each spectrum acquisition, a waveform generator (Hewlett-Packard, Palo Alto, Calif.) was triggered to release a burst of trigger pulses. Repetition rate and number of burst pulses determined the trapping and acquisition times, respectively. Each trigger pulse activated a delay generator (Stanford Research Systems, San Jose, Calif.) which in turn determined pulse widths and time delays in the electrostatic pulsing grids (e.g., an entrance grid and an exit grid). Output TTL signals from the delay generator were fed into two independent high-voltage switches (Behlke, Kronberg, Germany) that provided pulsed voltages for the two electrostatic pulsing grids. In continuous mode, the entrance grid was not pulsed and ESI-generated ions entered the trap continuously. Peptide samples were purchased (Sigma-Aldrich, St. Louis, Mo.), prepared in 50% aqueous methanol acidified with 1% acetic acid and used without further purification. Samples were infused into the mass spectrometer at a flow rate of 0.4 $\mu\text{L}/\text{min}$. The ion funnel and IFT were initially optimized by adjusting rf-fields and dc-fields in the trapping portion of the IFT for higher sensitivity. An optimum rf-amplitude was found for the trapping mode, although no significant signal variation was observed over a wide range of rf-amplitudes in continuous mode. 55 V_{p-p} was used as the optimal rf-amplitude, but is not limited thereto. Relationship for high m/z limit (m/z)_{high} as a function of the rf-frequency (f) and radial dc-electric field component (E_n) can be estimated as follows:

$$m/z_{\text{high}} = e V_{RF}^2 \exp(-2k_0/\delta) / 2 m_u \omega^2 \delta^3 E_n \quad (1)$$